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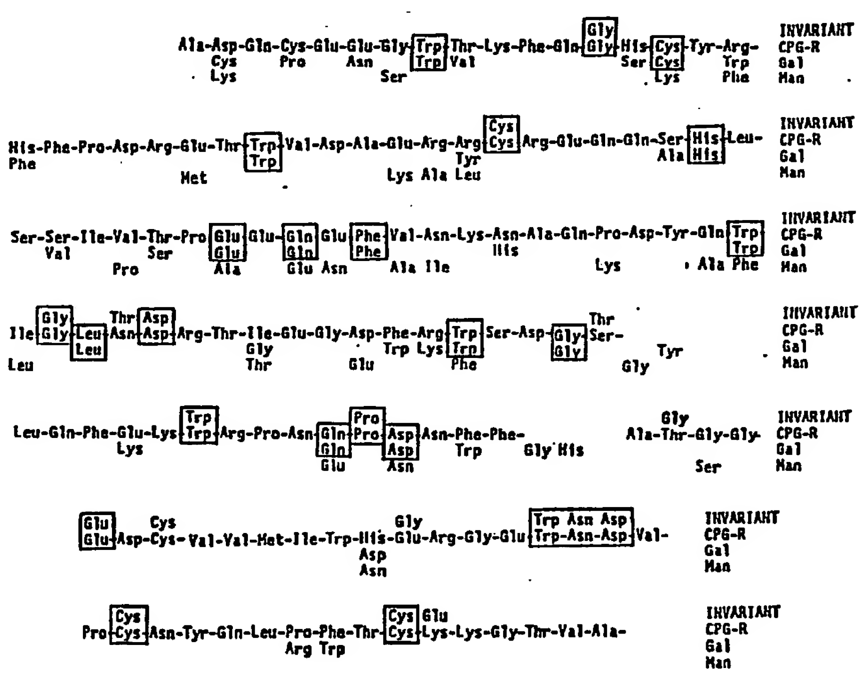

(57) Abstract

This invention provides nucleic acid engineered to produce peptides related to human mannose binding protein. These peptides are used in diagnosis and treatment of diseases.

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HUMAN MANNOSE BINDING PROTEIN

Background of the Invention

This invention relates to proteins able to bind mannose.

Mannose-binding proteins (MBPs) have been isolated from rabbit, rat, and human liver. Taylor et al. Clinical Science 70:539, 1986. MBPs have also been found in serum, and may play a role in the disposal of pathogenic organisms. Id.

Summerfield et al. Bioc. Biop. Acta 883:197, 1986 describe two types of MBP's in human serum. These were detected using antibodies raised against a 30 kDa subunit of one MBP. The authors suggest that MBPs may bind noxious glycoproteins in the circulation prior to the removal of these glycoproteins; and that yeasts and bacteria contain glycoproteins which and are bound by MBPs.

Stahl et al. Biol. Cell 51:215, 1984 describe a mannose receptor, which is distinct from MBPs. These two proteins appear to be structurally related since antibodies to one protein may react with the other protein.

Wild et al. Biochem. J. 210:167, 1983 describe the isolation of MBP from human and rat liver. The human MBP has a molecular weight greater than one million and consists of 28 kDa and 30.5 kDa subunits.

Drickamer et al. J. Biol. Chem. 261:6878, 1986 describe the isolation of MBPs from rat liver, and the cloning of cDNAs encoding these proteins. Each MBP has a cysteine rich region, a collagen-like domain and a carbohydrate binding domain.

Summary of Invention

In one aspect, the invention features engineered nucleic acid encoding for at least about 20 contiguous amino acids of human mannose-binding protein, or having at least about 60 or 90 bases able to hybridize under hybridizing conditions to nucleic acid encoding human mannose-binding protein. By engineered nucleic acid is meant nucleic acid removed from its natural environment by recombinant DNA methodology, or synthetic nucleic acid, or cDNA. This nucleic acid may be a fragment of DNA or RNA, it may be present in a vector system, or it may be within the genome of an organism.

The other aspects, the invention features vectors, and expression vectors or cells containing these vectors, each vector having the engineered nucleic acid, and the invention features peptides expressed from these vectors or cells. By peptide is meant a chain of two or more amino acids, including proteins and polypeptides. These peptides, and antibodies to these peptides, may be used as therapeutic or diagnostic agents.

In preferred embodiments, the nucleic acid encodes for a peptide having a greater than 75% homology to a fragment of at least thirty amino acids of human mannose-binding protein; most

preferably the nucleic acid encodes for human mannose-binding protein. In other preferred embodiments the nucleic acid is cDNA; the hybridizing conditions are at 42°C in 5 x SSC, with washing at 68°C in 0.1 x SSC; and the nucleic acid encodes a carbohydrate binding region. Most preferably the region has at least about 60 bases from region 309-714, shown in Fig. 1; the nucleic acid is ligated to nucleic acid encoding the toxic part of a toxin molecule, most preferably the toxin molecule is chosen from AZT, ricin, or cholera toxin; the cell is a virus, bacterium, fungus, or eucaryotic cell; the virus is vaccinia, the bacterium is Escherichia coli, the fungus is yeast, and the eucaryotic cell is a cultered cell line.

In a related aspect, the invention features a fragment of at least about 60 contiguous bases of the nucleic acid encoding human mannose binding protein deposited in the ATCC as strain number 67483.

In another aspect, the invention features a method for treating animals infected with a bacterium, fungus, or virus. The method entails providing a peptide able to bind the mannose units on these organisms. The peptide is able to cause host defensive cells to be attracted to the organisms. The method further entails administering the peptide to the animal.

In preferred embodiments, the peptide is a fragment of human mannose binding protein able to bind a carbohydrate; this peptide is able to disable the bacterium, fungus, or virus, and is a peptide as described above. Most preferably, the

animal is human; the infection results in a bacteremia or local bacterial infection, parasitic infection, or fungal colonization, and the route of administration is either intravenous, intramuscular, oral, or local, i.e., in the form of a powder, or lotion; or the virus is HIV or a related virus, and the peptide lowers the rate of infection of eucaryotic cells by the virus; the protein or peptide is the mannose binding protein provided at 1-500 µg/ml final concentration in human serum or tissue.

In another aspect, the invention features a method for diagnosing patients susceptible to invention by viruses, bacteria, parasites or fungi, the method features detecting the serum level of mannose-binding proteins in an animal, wherein this level reflects the susceptibility of the animal to an infection.

Preferably, the method features detecting reaction of an antibody to the above peptides with the serum, most preferably the detecting comprises an ELISA test.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Fig. 1 is a restriction endonuclease map of the MBP-human cDNA insert in pMBP.

Fig. 2 is a representation of the cDNA sequence and corresponding amino acid sequence of MBP human.

Fig. 3 is a representation of the genomic DNA, and corresponding amino acid sequences in all three reading frames of human MBP.

Fig. 4 is a diagram of a proposed model of MBP.

Fig. 5 is a comparison of the amino acid sequences of MBP-human with other lectins; invariant regions are shown on the top line, and galactose and mannose-specific regions on the lower lines.

Human Mannose-binding protein (MBP-human)

MBP-human is a soluble lectin-like molecule which is synthesized in hepatocytes and released into the bloodstream. Generally, MBP-human is able to bind carbohydrates, such as mannose, at its carbohydrate binding domain. MBP-human can be isolated generally as described by Wild et al., *supra*, and Drickamer et al. *supra*, for example, by passage down a mannose-sepharose column.

The general structure of MBP-human is shown in Fig. 4. The amino-terminal end 10 is cysteine rich, consistent with multimer formation by interchain disulfide bridges. Next to this is a collagen-like segment 12 having a repeated pattern of Gly-X-Y (Gly represents glycine; X and Y are other amino acids), similar to those of non-filbrillar collagen genes. Finally, there is a

carboxy-terminal carbohydrate recognition domain 14. The mannose-binding domain is within the region.

Nucleic acid, for example, DNA, encoding MBP-human can be isolated by standard techniques. For example, oligonucleotide probes specific for the nucleic acid may be constructed and used to probe either genomic or cDNA libraries, as described by Drickamer et al., <u>supra</u>. Altenatively, gene fragments from related genes can be used as probes. Preferably, the probe is homologous to a region of the carbohydrate binding domain of MBP-human. The clones isolated by this technique contain engineered nucleic acid. Once isolated, the gene encoding MBP-human is useful for producing recombinant MBP-human protein, or peptide fragments thereof. In addition, the nucleic acid can be modified by standard techniques in order to express modified peptides.

Examples of cloning MBP-Human encoding nucleic acid are given below. These examples are not limiting to the invention and one skilled in the art will recognize that there are many equivalent means for accomplishing similar results.

<u>Example 1: cDNA clones</u>

A human liver cDNA library was constructed in pKT218 by standard technique as described by Woods et al. <u>79</u> <u>Proc.</u> <u>Natl.Acad.</u> <u>Sci.</u> <u>USA.</u> <u>5661</u>, 1982. This library was probed using a gel purified radiolabelled rat MBP-C cDNA sequence digested with <u>Xho</u>I and <u>Eco</u>RI as described by Drickamer et al., <u>supra</u>. This probe was used

under non-stringent conditions to identify potentially useful clones. The filters were prehybridized for 1 hour at 42°C in 0.75 M NaCl, 50mM sodium phosphate, pH7.4, 5mM EDTA, 5x Dehardts solution and 0.1% SDS (5 x SSC), and then hybridized overnight at 42°C. The filters were washed at 45°C in 2xSSC for 30 minutes and then in 1xSSC for 30 minuteN n addition a λHEPG2 gt10 cDNA library plated in E. coli C600 was screened, as described by Kwiatkowski et al. 323 Nature 455, 1986.

Five clones, including pMBP, were isolated and their sequences determined by the method of Sanger et al. (74 Proc. Natl. Acad. Sci. USA 5463, 1977) using M13, Mp18 cloning vectors (Messing et al. Proc. Nat. Acad. Sci. USA 74:3642, 1977). This sequence is shown in Fig. 2. The restriction map of pMBP is shown in Fig. 1, it has a 3.6kb EcoRI insert isolated from the above λ gt 10 library.

Example 2: Genomic clone

The 650 bp carboxy terminal Pst-1 fragment (Fig. 1) of a MBP-human cDNA clone was used as a probe for human genomic library. This library was contructed by standard techniques in EMBL 3A by inserting Mbol-digested genomic DNA into the BamHI site. Clones which hybridized under stringent conditions were isolated. Specifically, the hybridization was performed as described above, except the wash conditions were at 68°C in 0.1xSSC.

The positively identified clones were plaque purified and their nucleic acid sequence determined as above. This sequence is presented in Fig. 3.

Other related genes can be isolated by this procedure. For example, the membrane receptor protein of macrophages is similar to MBP-human in that its DNA hybridizes under less stringent conditions (using the above hybridization buffer at 37°C) to MBP-human probes, and a peptide of similar size to MBP-human is immunoprecipitated with antisera to MBP-human.

Expression of MBP-human peptide fragments is by standard procedure. For example, the desired region of the MBP-human encoding DNA, preferably the cDNA, can be isolated from one of the above-described clones and inserted into any one of several standard expression vectors. A preferred region for expression is that encoding the carbohydrate binding domain, most preferably the mannose binding domain. This region is between nucleotide bases 359-807 in Fig. 1, including a 350bp Pst1-XbaI fragment. To identify the desired region more specifically the sequence is compared to that in related proteins such as human mannose receptor. Comparison to rat A and C MBPs reveals most homology between other mannose binding proteins at the region equivalent to the collagen region, at nucleotide bases 287-359 (Fig. 1). This region is not useful in the invention

since it is not involved with mannose binding. Rather, the region from 359-807 in Fig. 1 is most useful.

In order to show that any particular region of MBP-human does bind mannose the cDNA encoding it can be engineered by standard procedures to produce clones containing just this region. The resulting cloned DNA is then inserted into an expression vector. The peptide produced by such a vector is then passed through a mannose-sepharose column to see whether it will bind to mannose. Alternatively, a radioimmunoassay can be performed to see if radiolabelled mannose will react with the expressed peptide. Those peptides which bind mannose are useful in this invention.

It is unlikely that a single short linear region of amino acids of the MBP-human peptide is involved in binding to mannose, rather two or more such regions will probably cooperate to form a three-dimensional peptide configuration which can interact with, and bind, mannose. Such regions can be identified by comparison to other mannose-binding proteins as described above, and the DNA fragment encoding all such regions cloned and expressed. Such a DNA fragment is likely to be at least 60-90 base pairs in length, encoding at least about 20-30 amino acids.

Referring to Fig. 5, such a comparison was performed by comparing other lectins, with mannose or other sugar binding specifities, to MBP-human. The lower line of the figure shows a concensus for mannose binding proteins, the amino acids on this

line and in the upper line (showning invariant amino acids) are the most important for binding to mannose. These results were obtained by comparison of MBP-human to lectin proteins including the human and rat hepatic asialoglycoprotein receptors (Drickamer, 1987, Structure and biosynthesis of membrane receptors which mediate endocytosis of glycproteins, Kidney International, in press), the avian heptic receptor (Drickamer, 1987 supra), the apoprotein of dog (Benson et al., Proc. Natl. Acad. Sci. USA 82:6379, 1985) and human surfactant (White et al., Nature 317:361, 1985); the NH_2 portion of a glalctose specific lectin isolated from the hemolymph of *S. periginia* (Takahashi et al., J. Biol. Chem. 260:12228, 1985); a lectin isolated from the coelomic fluid of a sea urchin *A. crassispina* (Giga et al., J. Biol. Chem. 13: 6197, 1987); a chicken cartilage core proteoglycan protein (Shigaku et al., Proc. Natl. Acad. Sci. USA 83:5081, 1986) and the IgE Fc receptor (Ikuta et al., Proc. Natl. Acad. Sci. USA 84:819, 1987).

The above described mannose binding peptide, or the entire recombinant protein, is useful for specifically targeting cells expressing mannose on their surface, e.g., bacteria, fungi, and viruses. Thus, by linking this peptide to molecules able to kill or inhibit growth of such cells a hybrid peptide of great therapeutic use can be constructed. For example, the toxic part of ricin and cholera toxin, or chemicals such as AZT can be linked to this peptide. In order to do

this, the nucleic acid encoding such toxins can be ligated to the mannose binding peptide-encoding nucleic acid and expressed as a single entity to form a hybrid peptide, for example, as described by Murphy U.S. Patent 4,675,382, hereby incorporated by reference. Alternatively, the two peptides can be synthesized separately and linked chemically, for example, as described by Ross U.S. Patent 4,275,000, hereby incorporated by reference.

Expression vectors suitable for peptide expression include all standard bacterial (e.g., pKK233-2, Amann et al. Gene in press, sold by Pharmacia, 800 Centennial Avenue, Piscataway, NJ 08854), yeast, and viral expression vectors, as well as eucaryotic vectors. Those skilled in the art will realize that such vectors generally are suitable for expressing the protein and the example below is not limiting to this invention.

The full length or partial cDNA MBP clone, with or without toxin peptide-encoding nucleic acid, can be ligated into the vector pSV2neo (Southern et al. J. Mol. Appl. Genet. 1:327 (1982) and Cloning Vectors, A Laboratory manual Ed. Pouwels et al. Elsevier Science Pub. NY, 52 Vanderbilt Avenue, NY, NY 10017, 1985) which contains an origin of replication from pBR322 and an ampicillin resistance gene. It also contains SV40 sequences to provide a transcriptional promoter and a polyadenylation sequence. The DNA is inserted between these two sequences. After ligation the recombinant vector is propagated in

Escherichia coli and then introduced into Chinese hamster ovary cells using a standard calcium phosphate transfection protocol. The neo gene on this vector provides resistance to G418, which can be used to select for transformed cells.

Expression of mannose binding peptides by these vectors and organisms can be followed using a sepharose-mannose column. Expressed material is bound to the column, eluted with 50mM Tris/10mM EDTA, and run in 8% polyacrylamide gels (using Laemmli buffers, Nature 227:600, 1970) to observe the presence of peptides. Those clones which produce mannose-binding peptides, i.e., peptides which bind to such a column, are suitable in this invention.

Antibodies to such expressed peptides or to MBP-human itself can be produced by standard techniques. They may be monoclonal or polyclonal and are useful for identification of the peptides within animal serum or in clinical diagnostic tests.

Use

Exposed mannose is a feature of the cell walls of many pathogens, whereas higher organisms, including humans and animals, tend to have processed membrane glycoproteins having complex sugars which mask internal mannose residues. These internal mannose residues are not recognized by MBPs. Recombinant mannose binding protein, or chimeric peptides containing the mannose binding domain, are useful therapeutic agents. These proteins or peptides specifically bind

mannose-rich pathogens, including bacteria, fungi, yeasts, parasites, or the envelope glycoproteins of certain viruses, and thus direct removal of such pathogens from the animal.

For non-viral pathogens, efficacy of removal by host defense mechanisms may be increased by directing attachment of the mannose binding protein complex to the surface of phagocyte cells, thereby enhancing the clearance of the pathogens from the circulation, by causing the phagocytes to recognize these pathogens.

For viruses, which express mannose-rich glycoproteins, direct inactivation of the virus and viral infected cells is enhanced by attaching toxins, such as ricin, cholera, or diptheria or antimetabolite drugs, such as AZT, to the mannose binding domain of the mannose binding protein.

For example, the 350bp Pst1-XbaI fragment shown in Fig. 1, comprising the carboxy-terminal mannose binding domain of MBP-human can be expressed in an expression vector, and the peptide produced linked chemically to nucleotide analogues such as dideoxycytosine or AZT. As shown below, fluorescencely labelled such peptides do not bind to cells uninfected with HIV, the virus thought to cause Acquired Immunodeficiency Syndrome (AIDS), but do bind to infected cells. The resulting product should be particularly effective in specifically targeting drug-like molecules to HIV or HIV-infected cells.

Example 3: HIV targeting

MBP-human was shown to be effective *in vivo* for preventing infection of H9 $CD4^+$ cells with HIV. Purified HIV was incubated in the presence

or absence of highly purified homogenous MBP-human (prepared as described by Summerfield et al., Biochimica et Biop. Acta 883:197, 1986; Wild et al., Biochem. J. 210:167, 1983; Townsend et al., Biochem. J. 194:209, 1981; and Kawasaki et al., J. Biochem. 94:937, 1983). The treated virus was then incubated with H9 $CD4^+$ lymphocytes (which are primary targets for HIV infection) and 7 days later viral infectivity was measured by a) the appearance of HIV envelope glycoprotein (which was assayed on the cell surface by immunofluorescence using specific anti-envelope glycoprotein antisera) and b) the presence of reverse transcriptase activity (which is present only when the cell is infected with HIV). MBP-human completely inhibited viral entry into cells. This was shown by the absence of HIV envelope glycoprotein on the cell surface, and by indectectable reverse transcriptase activity. Control experiments showed that the inhibition by MBP-human was specific; these experiments involved completing MBP-human with mannose rich yeast mannan, and neo-glycoprotein mannose-BSA.

In experiments using fluorescently-labelled MBP-human to observe binding to infected or uninfected cells, the fluorescently labelled MBP-human was used to show that mannan and mannose-BSA inhibits the binding of MBP-human to virally infected cells, and that MBP-human does not bind uninfected H9 cells. Thus MBP-human is recognizing exposed mannose units on these cells.

Thus, MBP-human or the mannose binding domain thereof are suitable for identifying cells infected with HIV, or related viruses which

express mannose rich envelope glycoproteins on their cell surface. The MBP-human, the mannose binding domain or chimeric molecules thereof can be used to target cytotoxic agents to directly and specifically kill infected cells. Further, these molecules can be used to prevent the spread of viral infection, and even the initial infection itself.

MBP-human and related peptides as described above may be administered by routine methods. For example, they can be injected directly into the blood stream of an animal, especially humans, to a level of between 1-500µg/ml serum (most preferably, 150µg/ml final concentration, and this dose repeated to maintain this level. They can be administered prophylaticaly or after infection. Similarly, the molecules may be administered orally, injected subcutaneously, or even applied in powder or lotion form, for example, to treat local infections, such as bacterial infection, or infection with <u>Trichophyton</u> <u>rubrum</u>, which causes athlete's foot.

Another use of these peptides is in the determination of an animal's susceptibility to infection by agents such as HIV. Here, the serum level of MBPS in the animal is measured using antibodies produced to MBP-human, or related peptides, in for example, an ELISA protocol. The level of MBPs in the serum can then be related to the susceptibility to infection of this animal to an agent, and this relationship used to estimate other animals' susceptibility. Thus, for example if a high level of MBP-human is linked to low susceptibility to infection by HIV, then a human

having a low level of MBP-human is likely to be susceptible to HIV infection. Further, at the genomic level, such susceptibility may be related to defects in the nucleic acid. Such defects can be discovered using the cloned MBP-human genes, or fragments thereof, as probes. Polymorphisms linked to HIV susceptibility can be detected and used to predict susceptibility of other humans to infection.

Deposits

The following deposit was made on August 4, 1987, with the American Type Culture Collection (ATCC), where the deposit was given the accession number ATCC 67483.

Applicants' assignee, Children's Medical Center Corporation, represents that the ATCC is a depository affording permanence of the deposit and ready accessibility thereto by the public if a patent is granted. All restrictions on the availability to the public of the material so deposited will be irrevocably removed upon the granting of a patent. The material will be available during the pendency of the patent application to one determined by the Commissioner to be entitled thereto under 37 CFR 1.14 and 35 USC 122. The deposited material will be maintained with all the care necessary to keep it viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of the deposited microorganism, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of the patent, whichever period

is longer. Applicants' assignee acknowledges its duty to replace the deposit should the depository be unable to furnish a sample when requested due to the condition of the deposit.

Other embodiments are within the following claims.

Claims

1. Engineered nucleic acid encoding for at least about 20 contiguous amino acids of human mannose-binding protein.

2. Engineered nucleic acid comprising at least about 60 bases able to hybridize under hybridizing conditions to nucleic acid encoding human mannose-binding protein.

3. The nucleic acid of claim 1 or 2 wherein said nucleic acid encodes a peptide having greater than 75% homology to a fragment of at least thirty amino acids of said human mannose-binding protein.

4. The nucleic acid of claim 2 wherein said nucleic acid encodes said human mannose-binding protein.

5. The nucleic acid of claim 4 wherein said nucleic acid is cDNA.

6. The nucleic acid of claim 2 wherein said nucleic acid comprises at least about 90 bases able to hybridize under hybridizing conditions to human nucleic acid encoding mannose-binding protein.

7. The nucleic acid of claim 2 wherein said hybridizing conditions comprise 42°C in 5 x SSC, with washing at 68°C in 0.1 x SSC.

8. A nucleic acid fragment of at least about 60 contiguous bases of the nucleic acid encoding human mannose binding protein deposited in the ATCC as strain number 67483.

9. The nucleic acid of claim 6 wherein said nucleic acid encodes a carbohydrate binding region.

10. The nucleic acid of claim 9 wherein said region comprises at least about 60 bases from region 359-807 shown in Fig. 1.

11. The nucleic acid of claim 10 wherein said nucleic acid is ligated to nucleic acid encoding the toxic part of a toxin molecule.

12. The nucleic acid of claim 11 wherein said toxin moleucule is chosen from AZT, ricin, or cholera toxin.

13. A vector or an expression vector comprising the nucleic acid of claims 1, 2, 8, 9, 10, 11, or 12.

14. A peptide encoded by the nucleic acid of claim 1, 2, 8, 9, 10, 11, or 12.

15. A cell comprising the nucleic acid of claim 1, 2, 8, 9, 10, 11, or 12.

16. A cell comprising the peptide of claim 14.

17. The cell of claim 15 wherein said cell is bacterium, fungus, or eucaryotic cell.

18. The cell of claim 16 wherein said virus is vaccinia, said bacterium is Escherichia coli, said fungus is yeast, and said eucaryotic cell is a cultered cell line.

19. A therapeutic agent comprising the peptide of claim 14.

20. A method for treating animals infected with a bacterium, a fungus, or a virus,

said method comprising

administering to said animal a peptide able to bind to mannose.

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21. The method of claim 20 wherein said tide comprises a fragment of human mannose ding protein able to bind a carbohydrate.

22. The method of claim 21 wherein said tide inhibits growth or infection of said terium, fungus, or virus.

23. A method for treating animals infected a bacterium, a fungus, or a virus, said od comprising administering to said animal a tide of claim 14.

24. The method of claim 20 wherein said al is human.

25. The method of claim 24 wherein said tide is administered by application of a powder rising said peptide to said foot.

26. The method of claim 23 wherein said is is HIV and said peptide lowers the rate of ction of eucaryotic cells by said virus.

27. The method of claim 24 wherein said nistration is local, intravenous, amuscular or oral.

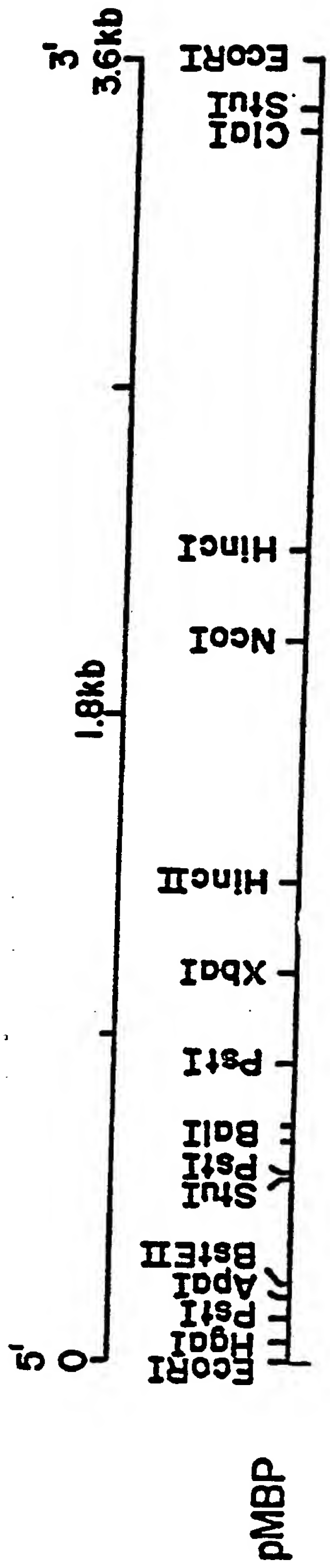
28. Antibodies binding to the peptide of m 14.

29. A method for diagnosing susceptibility nfection by viruses, bacteria, or fungi, said od comprising detecting the serum level of ose-binding proteins.

30. The method of claim 29 wherein said cting comprises detecting reaction of an body of claim 15 with said serum.

31. The method of claim 30, wherein said cting comprises of ELISA test.

FIG. 1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NcoI
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BalI
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ApaI
PstI
HgaI
EcoRI
pMBP

2/9

FIG. 2

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gct cgg taa ata tgt ttc att aac tga gat taa cct tcc ctg agt
ttt ctc aca cca agg gag acc ATG TCC TGT TTC ATC ACT CCC TCT
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Pro Cys Phe His Leu Pro Ser Ala Val Cys Glu Phe Pro Ile
                                                        855
agg gtc tgt gag ttc cct atc tga agg gtc ata tca ctc agg ccc
tcc ttg tct ttt tac tct acc aca gcc cac gta tgc ttg aaa gat
aat gat aat ata tca ttc tca gat cag tac ctg cag atg aaa ata
tca gat cag tac ctg cag atg aag ata aga cgg cat tta ttt ttc
cat tta caa caa aca cct gtg tgt tga gcc tta ctt tct gtt tgg
gta gag ggc tcc cct aat gac atg acc aca gtt taa tac cac agc
ttt ttt acc aag ttt cag gta tta aga aaa tct att ttg taa ctt
tct cta tga act ctg ttt tct ttc taa tga gat att aaa cca tgt
aaa aaa aaa aaa aaa aaa                                 993
```

FIG. 3

```
        10        20        30        40        50        60
CGCCAGAAAGTAGAGAGGTATTTAGCACTCTGCCAGGGCCAACGTAGTAAGAAATTTCCA
ArgGlnLysValGluArgTyrLeuAlaLeuCysGlnGlyGlnArgSerLysLysPhePro
 AlaArgLysEndArgGlyIleEndHisSerAlaArgAlaAsnValValArgAsnPheGl
  ProGluSerArgGluValPheSerThrLeuProGlyProThrEndEndGluIleSerA

        70        80        90       100       110       120
GAGAAAATGCTTACCCAGGCAAGCCTGTGTAAAACACCAAGGGGAAGCAAACTCCAGTTA
GluLysMetLeuThrGlnAlaSerLeuCysLysThrProArgGlySerLysLeuGlnLeu
nArgLysCysLeuProArgGlnAlaCysValLysHisGlnGlyGluAlaAsnSerSerEn
rgGluAsnAlaTyrProGlyLysProValEndAsnThrLysGlyLysGlnThrProValA

       130       140       150       160       170       180
ATTCTGGGCTGGGTTGGTGACTAAGGTTGAGGTTGATCTGAGGTTGAGACCTTCCTCTTT
IleLeuGlyTrpValGlyAspEndGlyEndGlyEndSerGluValGluThrPheLeuPhe
dPheTrpAlaGlyLeuValThrLysValGluValAspLeuArgLeuArgProSerSerLe
snSerGlyLeuGlyTrpEndLeuArgLeuArgLeuIleEndGlyEndAspLeuProLeuT

       190       200       210      220-       230       240
GGATCACCAGCTTTCAGCTCAGGGCCTGCCAATGAGTTTAAATGATAGTTAACAGGTCCT
GlySerProAlaPheSerSerGlyProAlaAsnGluPheLysEndEndLeuThrGlyPro
uAspHisGlnLeuSerAlaGlnGlyLeuProMetSerLeuAsnAspSerEndGlnValLe
rpIleThrSerPheGlnLeuArgAlaCysGlnEndValEndMetIleValAsnArgSerT

       250       260       270       280       290       300
GGAGGGGAATCAGCTGCCCAGATCAAAGATGGGATTCAGGTGGCAGATGGACCCGAAGAG
GlyGlyGluSerAlaAlaGlnIleLysAspGlyIleGlnValAlaAspGlyProGluGlu
uGluGlyAsnGlnLeuProArgSerLysMetGlyPheArgTrpGlnMetAspProLysAr
rpArgGlyIleSerCysProAspGlnArgTrpAspSerGlyGlyArgTrpThrArgArgG

       310       320       330       340       350       360
GACATGGAGAGAAAGAGGAAGAGGAAGCTCCTACAGACACCTGGGTTTCCACTCATTCTC
AspMetGluArgLysArgLysArgLysLeuLeuGlnThrProGlyPheProLeuIleLeu
gThrTrpArgGluArgGlyArgGlySerSerTyrArgHisLeuGlyPheHisSerPheSe
lyHisGlyGluLysGluGluGluAlaProThrAspThrTrpValSerThrHisSerH

       370       380       390       400       410       420
ATTCCCTAAGCTAACAGGCATAAGCCAGCTGGCAATGCACGGTCCCATTTGTTCTCACTG
IleProEndAlaAsnArgHisLysProAlaGlyAsnAlaArgSerHisLeuPheSerLeu
rPheProLysLeuThrGlyIleSerGlnLeuAlaMetHisGlyProIleCysSerHisCy
isSerLeuSerEndGlnAlaEndAlaSerTrpGlnCysThrValProPheValLeuThrA

       430       440       450       460       470       480
CCACGGAAAGCATGTTTATAGTCTTCCATGTTTATAGTCTTCCAGCAGCAACGCCAGGTG
ProArgLysAlaCysLeuEndSerSerMetPheIleValPheGlnGlnGlnArgGlnVal
sHisGlyLysHisValTyrSerLeuProCysLeuEndSerSerSerAsnAlaArgCy
laThrGluSerMetPheIleValPheHisValTyrSerLeuProAlaAlaThrProGlyV

       490       500       510       520       530       540
TCTAGGCACAGATGAACCCCTCCTTAGGATCCCCACTGCTCATCATAGTGCCTACCTTTG
SerArgHisArgEndThrProProEndAspProHisCysSerSerEndCysLeuProLeu
sLeuGlyThrAspGluProLeuLeuArgIleProThrAlaHisHisSerAlaTyrLeuCy
alEndAlaGlnMetAsnProSerLeuGlySerProLeuLeuIleIleValProThrPhe
```

FIG. 3 Contd

```
        550       560       570       580       590       600
TTAAAGTACTAGTCACGCAGGTTCACAAGGAATGTTTACTTTTCCAAATCCCCAGCTAGA
LeuLysTyrEndSerArgArgPheThrArgAsnValTyrPheSerLysSerProAlaArg
sEndSerThrSerHisAlaGlySerGlnGlyMetPheThrPheProAsnProGlnLeuGl
alLysValLeuValThrGlnValHisLysGluCysLeuLeuPheGlnIleProSerEndA

        610       620       630       640       650       660
GGCCAGGGTGGGTCATCTATTTCTATATAGCCTGCACCCAGATTGTAGGACAGAGGGCAT
GlyGlnGlyGlySerSerIleSerIleEndProAlaProArgLeuEndAspArgGlyHis
uAlaArgValGlyHisLeuPheLeuTyrSerLeuHisProAspCysArgThrGluGlyMe
rgProGlyTrpValIleTyrPheTyrIleAlaCysThrGlnIleValGlyGlnArgAlaC

        670       680       690       700       710       720
GCTCGGTAAATATGTGTTCATTAACTGAGATTAACCTTCCCTGAGTTTTCTCACACCAAG
AlaArgEndIleCysValHisEndLeuArgLeuThrPheProGluPheSerHisThrLys
tLeuGlyLysTyrValPheIleAsnEndAspEndProSerLeuSerPheLeuThrProAr
ysSerValAsnMetCysSerLeuThrGluIleAsnLeuProEndValPheSerHisGlnG

        730       740       750       760       770       780
GTGAGACCATGTCCTGTTTCATCACTCCCTCTCTTCTCCTGAGTATGGTGGCAGCGTCTT
ValArgProCysProValSerSerLeuProLeuPheSerEndValTrpTrpGlnArgLeu
gEndAspHisValLeuPheHisHisSerLeuSerSerProGluTyrGlyGlySerValLe
lyGluThrMetSerCysPheIleThrProSerLeuLeuLeuSerMetValAlaAlaSerT

        790       800       810       820       830       840
ACTCAGAAACTGTGACCTGTGAGGGTGCCCAAAAGACCTGCCCTGCAGTGATTGCCTGTA
ThrGlnLysLeuEndProValArgValProLysArgProAlaLeuGlnEndLeuProVal
uLeuArgAsnCysAspLeuEndGlyCysProLysAspLeuProCysSerAspCysLeuEn
yrSerGluThrValThrCysGluGlyAlaGlnLysThrCysProAlaValIleAlaCysS

        850       860       870       880       890       900
GCTCTCCAGGCATCAACGGCTTCCCAGGCAAAGATGGGCGTGATGGCACCAAGGGTAGAA
AlaLeuGlnAlaSerThrAlaSerGlnAlaLysMetGlyValMetAlaProArgValGlu
dLeuSerArgHisGlnArgLeuProArgGlnArgTrpAlaEndTrpHisGlnGlyEndLy
erSerProGlyIleAsnGlyPheProGlyLysAspGlyArgAspGlyThrLysGlyArgL

        910       920       930       940       950       960
AAGGGGGAACAGGTACGTGTTGGGCTGTTCTGTCTCTGCAATTCTTTACCTTCCAGAGGA
LysGlyGluGlnValArgValGlyLeuPheCysLeuCysAsnSerLeuProSerArgGly
sArgGlyAsnArgTyrValLeuGlyCysSerValSerAlaIleLeuTyrLeuProGluGl
ysGlyGlyThrGlyThrCysTrpAlaValLeuSerLeuGlnPhePheThrPheGlnArgL

        970       980       990      1000      1010      1020
AACTGCCTGGGGATATGAGGAGACGGATGTCCTATTTGAGTATATTTTTCTCAACTATAC
AsnCysLeuGlyIleEndGlyAspGlyCysProIleEndValTyrPheSerGlnLeuTyr
uThrAlaTrpGlyTyrGluGluThrAspValLeuPheGluTyrIlePheLeuAsnTyrTh
ysLeuProGlyAspMetArgArgArgMetSerTyrLeuSerIlePhePheSerThrIleL

       1030      1040
TGTAACTCAAAACAGAGATTCAGCTC
CysAsnSerLysGlnArgPheS r
rValThrGlnAsnArgAspSerAla
euEndLeuLvsThrGluIleGlnLeu
```

SUBSTITUTE SHEET

FIG. 3 Contd

```
          550       560       570       580       590       600
AAGTTGGGGCCTCCAGGAAATCCAGGGCCTTCTGGGTCACCAGGACCAAAGGGCCAAAAA
LysLeuGlyProProGlyAsnProGlyProSerGlySerProGlyProLysGlyGlnLys
uSerTrpGlyLeuGlnGluIleGlnGlyLeuLeuGlyHisGlnAspGlnArgAlaLysLy
ysValGlyAlaSerArgLysSerArgAlaPheTrpValThrArgThrLysGlyProLysA

          610       620       630       640       650       660
GGAGACCCTGGAAAAAGTCCGGGTAAGGACCCCAGCAAGGTCTGAGCTGACTTCACCCAG
GlyAspProGlyLysSerProGlyLysAspProSerLysValEndAlaAspPheThrGln
sGluThrLeuGluLysValArgValArgThrProAlaArgSerGluLeuThrSerProAr
rgArgProTrpLysLysSerGlyEndGlyProGlnGlnGlyLeuSerEndLeuHisProG

          670       680       690       700       710       720
GGTCCTGAGACCTTGAGTATCTGGTAAGAGGTGCCCCTTCTCCTGTTCCTTCAAAGGAAG
GlyProGluThrLeuSerIleTrpEndGluValProLeuLeuLeuPheLeuGlnArgLys
gValLeuArgProEndValSerGlyLysArgCysProPheSerCysSerPheLysGlyAr
lySerEndAspLeuGluTyrLeuValArgGlyAlaProSerProValProSerLysGluA

          730       740       750       760
ATACCCAAATTTGCTTTCTGACCCAGTGCCCTCAGCCCTCTC
IleProLysPheAlaPheEndProSerAlaLeuSerProLeu
gTyrProAsnLeuLeuSerAspProValProSerAlaLeu
spThrGlnIleCysPheLeuThrGlnCysProGlnProSer
```

FIG. 4

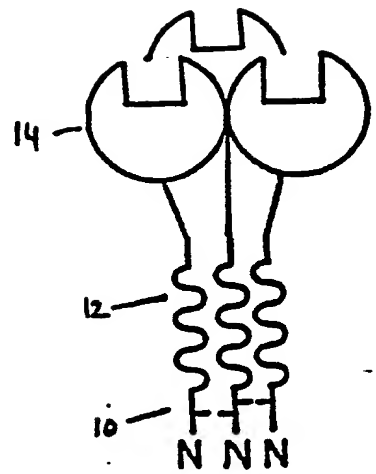

FIG. 5

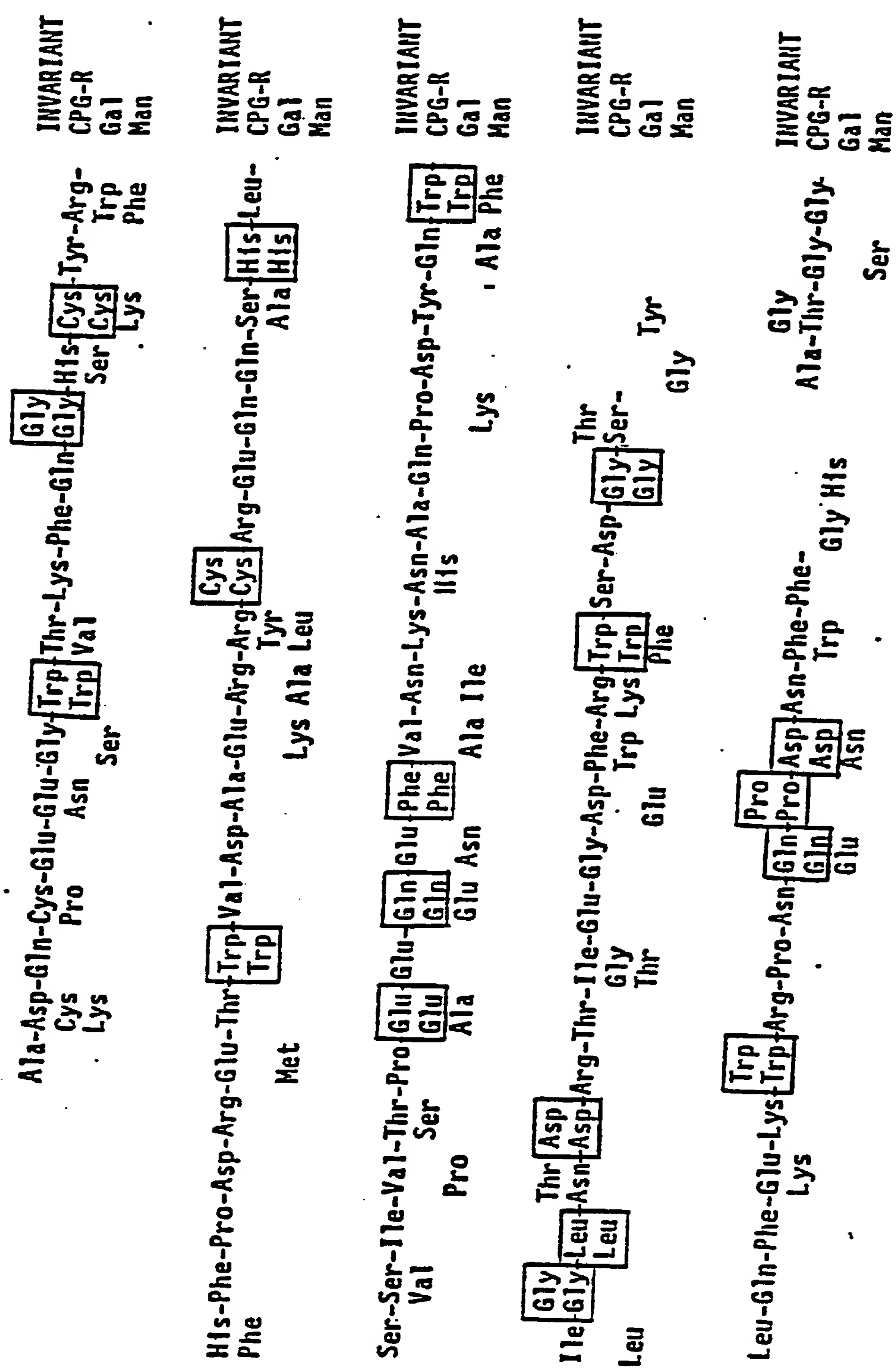
Ala-Asp-Gln-Cys-Glu-Glu-Gly-Trp-Thr-Lys-Phe-Gln-Gly-His-Cys-Tyr-Arg-
INVARIANT
CPG-R
Gal
Man
His-Phe-Pro-Asp-Arg-Glu-Thr-Trp-Val-Asp-Ala-Glu-Arg-Arg-Cys-Arg-Glu-Gln-Gln-Ser-His-Leu-
Ser-Ser-Ile-Val-Thr-Pro-Glu-Glu-Gln-Glu-Phe-Val-Asn-Lys-Asn-Ala-Gln-Pro-Asp-Tyr-Gln-Trp
Ile-Gly-Leu-Asn-Asp-Arg-Thr-Ile-Glu-Gly-Asp-Phe-Arg-Trp-Ser-Asp-Gly-Ser-
Leu-Gln-Phe-Glu-Lys-Trp-Arg-Pro-Asn-Gln-Pro-Asp-Asn-Phe-Phe-

FIG. 5 Contd

```
Glu      Cys                                    Gly                         Trp Asn Asp          INVARIANT
Glu-Asp-Cys- Val-Val-Met-Ile-Trp-His-Glu-Arg-Gly-Glu-Trp-Asn-Asp-Val-       CPG-R
                                  Asp                                                           Gal
                                  Asn                                                           Man

    Cys                                  Cys Glu                                                INVARIANT
Pro-Cys-Asn-Tyr-Gln-Leu-Pro-Phe-Thr-Cys-Lys-Lys-Gly-Thr-Val-Ala-                                 CPG-R
                            Arg Trp                                                              Gal
                                                                                                 Man
```

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 88/02591

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 N 15/00; 1/20; 1/14; 5/00; C 12 P 21/02; A 61 K 37/02; 39/395; G 01 N 33/569; //(C 12 P 21/00; C 12 R 1:91)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC[4]	C 12 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	Chemical Abstracts, volume 105, no. 21, 24 November 1986, (Columbus, Ohio, US), J.A. Summerfield et al.: "Mannose-binding proteins in human serum: identification of mannose-specific immunoglobulins and a calcium-dependent lectin, of broader carbohydrate specificity, secreted by hepatocytes", see page 573, abstract 189211v, & Biochim. Biophys. Acta 1986, 883(2), 197-206 cited in the application	14,28
A		29-31
	--	
A	The Journal of Biological Chemistry, volume 261, no. 15, 25 May 1986, The American Society of Biological Chemists, Inc., (Baltimore, US), K. Drickamer et al.: "Mannose-binding proteins isolated from rat liver contain carbohydrate-recognition domains linked to ./.	1-6,9,13-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th December 1988	11 JAN 1989
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
	collagenous tails", pages 6878-6887 see abstract; figure 10 cited in the application	
A	The Journal of Biological Chemistry, volume 262, no. 6, 25 February 1987, The American Society of Biological Chemists, Inc., (Baltimore, uS), K. Drickamer et al.: "Exon structure of a mannose-binding protein gene reflects its evolutionary relationship to the asialoglycoprotein receptor and nonfibrillar collagens", pages 2582-2589 see abstract	1-6,9,13-17
P,A	The Journal of Biological Chemistry, volume 262, no. 29, 15 October 1987, The American Society for Biochemistry and Molecular Biology, (Baltimore, US), H.P. Haagsman et al.: "The major lung surfactant protein, SP 28-36, is a calcium-dependent, carbohydrate binding protein", pages 13877-13880 see abstract; page 13879, column 2, lines 20-44	1-6,9,13-17,19
A	Nature, volume 317, 26 September 1985, (London, GB), R. Tyler White et al.: "Isolation and characterization of the human pulmonary surfactant apoprotein gene", pages 361-363 see abstract; figure 2 cited in the application	1-6,9,13-17
P,X	Chemical Abstracts, volume 109, no. 15, 10 October 1988, (Columbus, Ohio, US), R. Ezekowitz et al.: "A human mannose-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins", see page 268, abstract 124567d, & J. Exp. Med. 1988, 167(3), 1034-46	1-6,9,13-17
P,A	Chemical Abstracts, volume 109, no. 11, 12 September 1988, (Columbus, Ohio, US), R. Ezekowitz et al.: "The role of human mannose-lectin-like molecules in host defense", see page 517, abstract 90799p, & Symp. Mol. Cell. ./..	19

International Application No. PCT/US 88/02591

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

	New Ser. 1988, 64(Bact.-Host. Cell Interact.), 213-23 --	
A	WO, A, 83/03971 (PRESIDENT AND FELLOWS OF HARVARD COLLEGE) 24 November 1983 see claims cited in the application	11,12

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers ...*..... because they relate to subject matter not required to be searched by this Authority, namely:

* claims 20-27
See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

claim 18 was not searched because there is no meaningful role given for vaccinia virus.

2. [ ] Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers.........., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8802591

SA 24331

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 04/01/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8303971	24-11-83	AU-A-	1706283	02-12-83
		EP-A,B	0108146	16-05-84
		CA-A-	1217156	27-01-87
		US-A-	4675382	23-06-87
		AU-B-	573529	16-06-88

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82